RESEARCH NOTE

DOES TRANSOVARIAL TRANSMISSION OF DENGUE VIRUS OCCUR IN MALAYSIAN *AEDES AEGYPTI* AND *AEDES ALBOPICTUS?*

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Dengue is a disease of great public health importance in many tropical areas of the world, causing considerable morbidity and in some regions, significant mortality (WHO 1975; Halstead 1980). It is caused by four antigenically related virus serotypes which are dengue type 1, 2, 3 and 4 virus. The principal vector of dengue virus in urban areas is the highly domesticated *Aedes aegypti*, whereas *Ae. albopictus* is an important vector in some rural areas. Both species can breed in artificial and natural containers. In Southeast Asia, dengue epidemics usually take place during the rainy months and are correlated with increased vector populations and bleeding habitats (WHO, 1975). The cyclic nature of dengue epidemics and how the virus is maintained during interepidemic periods in areas where epidemics have occurred previously pose questions which have led to studies to evaluate the importance of transovarial transmission in dengue virus maintenance. Transovarial transmission of all four dengue serotypes in mosquitos has been demonstrated experimentally. The mosquito species in which dengue virus has been transovarially transmitted are *Ae. albopictus* (Mitchell and Miller, 1990; Rosen *et al*, 1985; Rosen, 1988), *Ae. aegypti* (Chen *et al*, 1990; Rosen *et al*, 1985), *Ae. mediovittatus* (Freir and Rosen, 1988), *Ae. alcasidi, Ae. cooki, Ae. herbrideus, Ae. katherinensis, Ae. malayensis, Ae. polynesiensis, Ae. pseudoscutellaris* and *Ae. tongae tabu* (Freir and Rosen, 1987).

Information on the ability of the local vectors to transmit dengue virus transovarially will be useful in assisting the public health personnel and the general public in implementing a more effective campaign against dengue and its vectors; for if transovarial transmission of dengue virus is confirmed, the control of the immature stages of *Aedes* mosquito and the elimination of breeding sources must be further emphasized and prioritized. This study was therefore initiated to investigate the possibility of such mechanism for dengue virus transmission in two Malaysian species of dengue vectors, namely, *Ae. aegypti* and *Ae. albopictus.*

Malaysian strains of *Ae. albopictus* and *Ae. aegypti* were used for the experiment. Both strains were maintained in Division of Medical Entomology, Institute for Medical Research, Kuala Lumpur and are free of dengue infection. Mosquitos were reared at ambient temperature and relative humidity. Eggs laid by the starting mosquito generations were pooled according to species, hatched, and the immature instars were reared to adults. The females (4-5 days old) were collected and membrane-fed with infectious blood meal. A second feeding, with normal blood, was given 7 days later when they were 11-12 days old. Only fully engorged females were collected and placed in separate cages for egg laying. Two batches of eggs from the different ovarian cycles were collected from each species with each batch of eggs collected until the 5th day after each blood meal. The two batches of F-2 eggs of the starting colony of each species were reared separately to the fourth instar larvae and pooled for virus isolation. All four serotypes of dengue virus were employed. They were originally obtained from human sera and stored in the Division of Virology, Institute for Medical Research, Kuala Lumpur; and had been passaged once in *Ae. albopictus* C6/36 cell cultures prior to the experiment. Pools of larvae were ground in chilled eppendorf tubes with 1.5 ml of a growth medium (Eagle's minimum essential medium, MEM), supplemented with 5% fetal bovine serum (FBS), 0.2 mM of non-essential amino acids and antibiotics. The mosquito suspensions were then centrifuged at 14,000 rpm for 15 minutes at 4°C and the mosquito supernatants were used for virus isolation in culture tubes with C6/36 cell monolayers (Maneekarn *et al*, 1993). The presence of dengue virus was detected by peroxidase antiperoxidase (PAP) staining (Ma-

neekarn *et al*, 1993). To further confirm the presence of dengue virus, mosquito supernatants were subjected to reverse-transcriptase polymerase chain reaction (RT-PCR) (Maneekarn *et al*, 1993). Briefly, cell culture fluid (5 µl) was treated with 5 µl of a mixture containing 1% Nonidet P-40 and 10 units of RNase inhibitor in PBS for 1 minute at room temperature. This was followed by addition of 90 µl RT-PCR mixture containing 100 pmol of each universal primer (TCAATATGCTGAAACG-CGCGAGAAACCG and TTGCACCAACAGT-CAATGTCTTCAGGTTC), 0.2 mM each of deoxynucleoside triphosphate (dATP, dCTP, dGTP, dTTP), 10 mM Tris (pH 8.9), 1.5 mM $MgCl_2$, 80 mM KCl, 0.5 mg/ml bovine serum albumin, 0.1% sodium cholate, 0.1% Triton X-100, 10 units of reverse transcriptase and 2 units of Tth DNA polymerase. The reaction mixture was overlayered with 2 drops of mineral oil and each tube was placed in a Perkim-Elmer-Cetus thermal cycler which was programed to incubate at 53°C for 10 minutes (for reverse transcription) followed by 35 cycles of: denaturation at 94°C for 1 minute, annealing at 53°C for 1.5 minutes and extension at 72°C for 2 minutes. After amplification, 15 µl of PCR product was electrophoresed on 3% agarose, stained with ethidium bromide, visualized under UVL and photographed.

None of the 16 pools (480 individuals) of *Ae. aegypti* and 14 pools (420 individuals) of *Ae. albopictus* L4 reared from the first batch of eggs were positive with dengue virus (Table 1). However, six of 13 pools (390) individuals) of *Ae. aegypti*, and none of 15 pools (450 individuals) of *Ae. albopictus* reared from the second batch of eggs were tested positive with dengue virus. Minimum filial infection rate (MFIR) for the *Ae. aegypti* L4 was 1:65 (Table 2). Pooled specimens were confirmed positive with dengue virus after a second passage in cell culture. For the purpose of comparison, culture fluids of 2 pools diagnosed positive by PAP staining were also re-confirmed by RT-PCR.

Thus the transovarial transmission of dengue virus was demonstrated in *Ae. aegypti*. Despite the fact that transovarial transmission of dengue virus was not demonstrated by the strain of *Ae. albopictus*, results of the negative test should be considered inconclusive. Studies have shown that transovarial transmission of dengue virus by *Ae. albopictus* varied extensively depending on the strain of virus and geographic strain of mosquito (Rosen *et al*,

Table 1

Minimum filial infection rates (MFIR) for *Aedes aegypti* and *Ae. albopictus* fourth instar larvae (L4) from the first ovarian cycle of females fed on a dengue virus infected blood meal.

Species	No. L4 examined	Pools (Positive/total)	MFIR
Aedes aegypti	480	0/16	< 1:480
Ae. albopictus	420	0/14	< 1:420

Table 2

Minimum filial infection rate (MFIR) for *Aedes aegypti* and *Ae. albopictus* fourth instar larvae (L4) from the second ovarian cycle of females fed on a dengue virus infected blood meal.

Species	No. L4 examined	Pools (Positive/total)	MFIR
Aedes aegypti	390	6/13	1:65
Ae. albopictus	450	0/15	< 1:450

1985). The demonstration of transovarial transmission of dengue virus in the strain of *Ae. aegypti* suggests that *Ae. aegypti*, the principal vector species in urban areas, may play a significant role in the maintenance of dengue virus in nature, in the absence of susceptible hosts or when climatic conditions are unfavorable for mosquito activity. The presence of transovarial dengue virus in wild *Aedes* larvae were recently detected by Rohani *et al* (1996). Adults of both species originated from 6 pools of field-collected larvae were found positive for dengue virus by the tissue culture method and out of these pools, 3 pools were reconfirmed by the RT-PCR method. Of the 6 positive pools, only 1 pool came from larvae of *Ae. aegypti*, the remaining pools being *Ae. albopictus*. The possibility of the transovarial transmission of dengue virus is further enhanced by the detection of the virus in the male *Ae. albopictus* originated from field-collected larvae. Thus, *Ae. albopictus* is also important as a maintenance host in the field.

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